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Description

METHOD AND APPARATUS FOR PRECISE TEMPERATURE CYCLING IN CHEMICAL/BIOCHEMICAL PROCESSES

Background of Invention

[0001] The present invention relates generally to temperature control systems, and, more particularly, to a method and apparatus for precise temperature cycling in chemical/biochemical processes, such as nucleic acid amplification, DNA sequencing and the like.

[0002] Polymerase Chain Reaction (PCR) is a chemical amplification technique developed in 1985 by Kary Mullis, in which millions of copies of a single DNA fragment may be replicated for use in research or forensic analysis. PCR involves three basic steps, each of which is performed at a specific temperature. To be most effective, these temperature changes should be as rapid as possible. In the first step, denaturing, a test tube containing the fragment is heated to about 95°C for a few seconds, thereby causing the dou-

ble-stranded DNA fragment to separate into two single strands. The second step is annealing, in which the temperature of the test tube is then lowered to about 55°C for a few seconds, causing primers to bind permanently to their sites on the single-stranded DNA. The third step is extending, in which the temperature is raised to about 72°C for about a minute, which causes the polymerase protein to go to work.

[0003] The protein moves along the single-stranded portion of the DNA, beginning at a primer, and creates a second strand of new DNA to match the first. After extension, the DNA of interest is double-stranded again, and the number of strands bearing the sequence of interest has been doubled. These three steps are then repeated about 30 times, resulting in an exponential increase of up to a billion-fold of the DNA of interest. Thus, a fragment of DNA that accounted for one part in three million, for example, now fills the entire test tube.

[0004] In conventional PCR equipment, an array of tubes or vials holding samples of DNA is placed in a metal block, and the temperature of the samples is controlled by heating and cooling the block. An alternative apparatus involves the use of a rapid thermal cycler, wherein samples are

placed in a plastic plate having water circulating underneath to set the temperature of the samples. In order to change the temperature of the samples in such a device, water is switched from one tank to another.

[0005] However one disadvantage of such existing PCR heating devices is the large thermal budget needed to heat the metal block or water. In addition, precise temperature control issues may also present a problem in that physical heat transfer mechanisms (e.g., conduction, convection) are needed to transfer heat from the metal block/water to the container, and then to the cultures themselves. Still another concern related to conventional heating equipment relates to the lag time associated with a change in temperature settings.

[0006] Accordingly, it would be desirable to implement a more precise heating system for chemical and biochemical uses, such as performing PCR.

Summary of Invention

[0007] The foregoing discussed drawbacks and deficiencies of the prior art are overcome or alleviated by a method for implementing a temperature cycling operation for a biochemical sample to be reacted. In an exemplary embodiment, the method includes applying an infrared (IR) heat-

ing source to the sample at a first infrared wavelength selected so as to generate a first desired temperature for a first duration and produce a first desired reaction within the sample. Following the first desired reaction, applying the infrared (IR) heating source to the sample at a second infrared wavelength selected so as to generate a second desired temperature for a second duration and produce a second desired reaction within the sample.

[0008] In another embodiment, a method for implementing temperature cycling for a polymerase chain reaction (PCR) process includes subjecting a DNA fragment to infrared radiation so as to facilitate at least one of a denaturing step, an annealing step and an extending step.

[0009] In still another embodiment, a temperature cycling apparatus includes a processing chamber and an infrared (IR) heating source. The infrared heating source is configured to generate energy a first infrared wavelength so as to generate a first desired temperature for a first duration and produce a first desired reaction within a sample placed in the processing chamber. The infrared (IR) heating source further is configured to generate energy at a second infrared wavelength so as to generate a second desired temperature for a second duration and produce a

second desired reaction within the sample.

Brief Description of Drawings

[0010] Referring to the exemplary drawings wherein like elements are numbered alike in the several Figures:

[0011] Figure 1 is a schematic illustration of a resonant, infrared reaction chamber, suitable for use in accordance with an embodiment of the invention;

[0012] Figure 2(a) is a graph illustrating a method for implementing a temperature cycling operation for a biochemical sample to be reacted, in accordance with an embodiment of the invention;

[0013] Figure 2(b) is a graph illustrating a method for implementing a temperature cycling operation for a biochemical sample to be reacted, in accordance with an alternative embodiment of the invention; and

[0014] Figure 3 is a schematic illustration of a method for implementing a continuous, temperature cycling batch operation for a biochemical sample to be reacted, in accordance with still another embodiment of the invention.

Detailed Description

[0015] Disclosed herein is a method and apparatus for precise temperature cycling in chemical/biochemical processes

(e.g., PCR), in which infrared (IR) resonant heating is used to selectively heat a chemical/biochemical culture. When electromagnetic (EM) radiation resonates at the natural vibrational frequency of a bond of a molecule in the material to which the EM energy is applied, the energy is absorbed and is manifested as heating, as a result of an increased amplitude of vibration. The resonant heating therefore enhances specificity of reactions, since only the desired molecules are directly heated by application of specific wavelengths of the EM radiation. With a large number of vibrational modes available for any given asymmetric surface species, resonance at a specific IR wavelength can be exploited to heat only the desired component. As a result, the application of selective resonant heating can effectively heat specific bonds to a desired temperature, thus attaining a much higher desired fractional dissociation relative to existing heating mechanism, without undesirable side reactions.

[0016] Moreover, since IR radiation heats the samples directly without heating the medium in between, this results in a fast, one-stage heat transfer that can conceivably lower the PCR cycle time from about 2-3 minutes, to possibly to a few seconds. Furthermore, since only the bonds of in-

terest are activated by the IR radiation, the effects of heating a metal/fluid or sample vials do not come into play, thereby lowering the overall thermal budget.

[0017] Although the embodiments described hereinafter are presented in the context of the PCR process, it should be appreciated that this process has been chosen herein as just one example to highlight the advantages of the IR resonant heating apparatus. As such, the present invention embodiments are not to be construed as being specifically limited to the PCR process, but rather can be applied to a broad range of chemical/biochemical systems and processes.

[0018] Referring initially to Figure 1, there is shown a schematic illustration of a resonant, infrared reaction chamber 100, suitable for use in accordance with an embodiment of the invention. The chamber 100 is configured to receive a plurality of specimen vials 102 therein, such as DNA fragment containing test tubes for PCR amplification, for example. A plurality of infrared radiation generation sources 104 are also included for providing EM radiation at one or more specifically desired wavelengths, such as in the Near IR or Mid IR bands. The IR sources may be obtained from any commercially available source, and preferably provide

a broad range of spectral radiance (e.g., 1-1000 W/cm^2).

[0019] In a temperature cycling process, such as the three-step process involved in PCR, the chamber 100 is configured to apply specifically targeted IR wavelengths to the vial contents in order to produce the three distinct reactions that take place at the different temperature values specified above. Thus, as shown in Figure 2(a), once the vials are placed within the chamber 100 (at about ambient temperature), they are initially subjected to a first IR wavelength (IR_1) specifically selected to carry out the denaturing step at about 95°C for about 30 seconds to separate the DNA into single strands. Then, the samples are subjected to a second IR wavelength (IR_2) specifically selected to carry out the annealing step at about 55°C for about 30 seconds for the primers to bind to the sites on the single strands. Finally, the samples are subjected to a third IR wavelength (IR_3) specifically selected to carry out the extending step at about 75°C for about a minute, where the polymerase protein creates new DNA to match the original.

[0020] In an alternative embodiment, a three-step temperature cycling process may be performed using two IR energy wavelengths. As depicted by the graph in Figure 2(b), the process chamber is initially heated and kept at a tempera-

ture representing the lowest of the three desired temperature values (in this example, 55°C). Thus, to implement the PCR process, the vials are initially subjected to the first IR wavelength (IR_1) for denaturing. Then, because the chamber is already heated to a baseline temperature of 55°C, no IR radiation is applied for a duration representing the completion time of the annealing step. In other words, the second IR wavelength (IR_2) used in the embodiment of Figure 2(a) is not used. Then, after the vials are exposed to the preheated annealing temperature for the requisite time, third IR wavelength (IR_3) is applied to the vials for the extending step.

[0021] Still a further embodiment of a precise temperature cycling method and apparatus is shown in Figure 3. As is shown, the system 300 can also be designed to conduct a batch operation in a continuous mode. Instead of using a single processing chamber with an infrared heating source of varying wavelengths, the samples 102 are exposed to IR radiation at specified wavelengths in discrete chambers 302a, 302b, 302c, by traveling along conveyor 304. Again, using the PCR example, the first chamber will include IR generation sources 104a configured for directing IR energy at the first IR wavelength (IR_1); the second

chamber will include IR generation sources 104b configured for directing IR energy at the second IR wavelength (IR_2); and the third chamber will include IR generation sources 104c configured for directing IR energy at the third IR wavelength (IR_3). This embodiment thus allows for higher throughput as the industry prepares to meet growing needs in the near future.

[0022] As will be appreciated from the above described embodiments, certain disadvantages of existing thermal cyclers used in the art (e.g., such as those having sample vials of DNA placed in either a metal block or in wells in a plastic plate with circulating fluid) are overcome, since the temperature of the samples is not controlled by the temperature of a metal block or circulating heating oil. As a result, thermal resistance issues emanating from conductive/convective heat transfer from a metal/fluid to polypropylene vials and then to the sample are avoided by the use of IR resonant heating.

[0023] Sample throughput may thus be increased due to a decreased lag time as a result of the time needed to change the cycle temperature settings in view of thermal resistances. Furthermore, the above described embodiment can alleviate the possibility of cross-reactivity with non-

targeted DNA sequencing that could otherwise result in non-specific amplification and primers reacting with one other.

[0024] While the invention has been described with reference to a preferred embodiment or embodiments, it will be understood by those skilled in the art that various changes may be made and equivalents may be substituted for elements thereof without departing from the scope of the invention. In addition, many modifications may be made to adapt a particular situation or material to the teachings of the invention without departing from the essential scope thereof. Therefore, it is intended that the invention not be limited to the particular embodiment disclosed as the best mode contemplated for carrying out this invention, but that the invention will include all embodiments falling within the scope of the appended claims.

[0025] What is claimed is: